REMARKS

Status of the Claims

Claims 4-10 and 14 have been canceled without prejudice or disclaimer as being drawn to non-elected subject matter. In view of amendments to claim 1, claim 13 has been canceled herein without prejudice or disclaimer. Applicants expressly reserve the right to file a divisional application or take other such appropriate action to seek protection of the subject matter encompassed by these canceled claims.

Claims 1, 2, and 3 have been amended herein. Specifically, claim 1 has been amended herein to recite that an NSO cell culture lacks foetal calf serum (FCS). Support for this amendment can be found in the specification at, for example, page 4, lines 19-20, and page 6, lines 5-9 and 18-22.

Claim 2 has been amended to recite that culturing NSO cells in the presence of the peptide-free sLDL particles increases NSO cell proliferation by at least 20% relative to NSO cells cultured in the absence of peptide-free sLDL particles and in the presence FCS or other serum-free lipid supplements. Claim 3 has been amended herein to recite that culturing NSO cells in the presence of sLDL particles comprising a peptide increases NSO cell proliferation by at least 50% relative to NSO cells cultured in the absence of such sLDL particles and in the presence of FCS or other serum-free lipid supplements. Support for these amendments can be found in the specification at, for example, page 6, lines 5-9.

No new matter has been added by way of these amendments; their entry into this application is therefore respectfully requested.

Claims 1-3, 11, and 12 are now pending. In view of the above amendments and the remarks below, Applicants respectfully request reconsideration and allowance of the pending claims. The Examiner's comments in the Office Action are addressed below in the order set forth therein.

The Rejection of the Claims as Being Indefinite Should Be Withdrawn

Claims 2 and 3 were rejected under 35 U.S.C. §112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, claims 2 and 3 were rejected as lacking a

reference or comparison by which an increase in cell numbers is measured. Claims 2 and 3 were further rejected for reciting the term "enable" which, according to the Examiner, is vague.

Without acquiescing to the Examiner's rejection, and solely to advance prosecution, Applicants have herein amended claims 1, 2, and 3. Amended claim 1, from which claims 2 and 3 depend, recites that the NSO cell culture lacks foetal calf serum (FCS). As amended, claim 2 recites that culturing NSO cells in the presence of the peptide-free sLDL particles increases NSO cell proliferation by at least 20% relative to NSO cells cultured in the absence of peptide-free sLDL particles and in the presence FCS or other serum-free lipid supplements. Similarly, amended claim 3 recites that culturing NSO cells in the presence of sLDL particles comprising a peptide increases NSO cell proliferation by at least 50% relative to NSO cells cultured in the absence of such sLDL particles and in the presence of FCS or other serum-free lipid supplements.

Applicants respectfully submit that one having ordinary skill in the art would understand that an increase in NSO cell proliferation of at least 20% and 50% in claims 2 and 3, respectively, is determined relative to NSO cell proliferation in cell culture samples containing foetal calf serum or other serum-free lipid supplements instead of the recited sLDL particles. Thus, the skilled artisan would readily appreciate that the claims are directed to use of the sLDL particles of the present invention in an NSO cell culture lacking FCS to significantly increase NSO cell proliferation relative to an NSO cell culture comprising FCS alone or other serum-free lipid supplements. Accordingly, Applicants submit that one having ordinary skill in the art would understand the metes and bounds of amended claims 1-3.

In light of the above, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

The Rejection of the Claims as Being Anticipated Should Be Withdrawn

Claims 1-3 and 11-12 were rejected under 35 U.S.C. § 102 for being anticipated by Baillie *et al.* (*J. Lipid Res.*, 2002, 43(1):69-73). This rejection is respectfully traversed with respect to the amended claims.

As described above, claims 1-3 have been amended to clarify that the claimed methods of

proliferating eukaryotic NSO cells involve NSO cell cultures lacking FCS, where culturing NSO cells in the presence of the recited sLDL particles increases NSO cell proliferation by at least 20% (claim 2) and at least 50% (claim 3) relative to NSO cell cultures supplemented with FCS or other serum-free lipid supplements and no sLDL particles.

Applicants respectfully note that a claim is anticipated only if each and every element as set forth in the claim is disclosed, either expressly or inherently, in a single prior art reference. MPEP § 2131 (citing *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987)). Applicants submit that the Baillie *et al.* reference fails to meet this standard as it does not disclose methods for increasing proliferation of NSO cells in culture. The methods of Baillie *et al.* are limited to proliferating U937 cells that are unable to perform *de novo* cholesterol synthesis and can only overcome this deficiency via the LDL-receptor-mediated uptake pathway. See the Baillie *et al.* reference at page 70, left column. As there is no disclosure of NSO cells, the Baillie *et al.* reference does not disclose each and every limitation of the present claims and does not anticipate the presently claimed methods of proliferating NSO cells.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-3 and 11-12 under 35 U.S.C. § 102(b).

Claims 1 and 13 were rejected under 35 U.S.C. § 102 for being anticipated by Gorfien *et al.* (*Biotech. Press*, 2000, 16(5):682-687). Specifically, it was asserted that the cited reference discloses adding a synthetic low density lipoprotein particle to a cell culture to increase proliferation of NSO cells. Claim 13 has been canceled as noted above. This rejection is respectfully traversed with respect to amended claim 1.

As described above, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. MPEP § 2131 (citing *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987)). Claim 1 is directed to methods of proliferating eukaryotic NSO cells, where the methods include introducing a synthetic low density lipoprotein (sLDL) particle to an NSO cell culture. As defined in the specification, a sLDL particle of the present invention comprises a lipid component comprising a lipid emulsion comprising a core of lipophilic molecules.

Gorfien *et al.* disclose methods of culturing NSO cells with a cholesterol supplement

consisting of methyl-β-cyclodextrin and cholesterol. See "Additive Options" section on page 683, left-hand column of this cited reference. As NSO cells are sterol auxotrophs, meaning they require a source of cholesterol in the cell culture medium, Gorfien *et al.* attempt to replace foetal bovine serum, the traditional source of cholesterol, with a cyclodextrin-cholesterol supplement. This supplement consists of animal-derived cholesterol or a plant-derived sterol that is complexed with cyclodextrin, the latter being a cyclic oligosaccharide that has a toroidal structure. The toroidal structure of cyclodextrin allows it to host hydrophobic molecules, such as cholesterol, leading to substantial improvements in water solubility. Increased solubility leads to improved bioavailability in cell culture and enables the supplement to be manufactured at a high concentration for dilution in the cell culture medium and greatly improves filterability (for sterilisation). This product is assimilated passively; there is no active LDL receptor-mediated uptake.

By contrast, synthetic low density lipoprotein (sLDL) of the present invention is a non-naturally occurring spherical lipid particle that mimics natural LDL in composition and function. As defined in the specification, the particle consists of a lipid emulsion comprising a core of lipophilic molecules such as cholesterol esters, triglycerides, etc. The lipophilic core is solubilized by means of a monolayer of an amphiphlic lipid, such as a phospholipid, bearing a hydrophilic group. sLDL does not require the inclusion of any additional solubilizer such as cyclodextrin. Accordingly, the cyclodextrin-cholesterol supplement of the Gorfien *et al.* reference is not encompassed by Applicants' definition of "sLDL particle," and there is no disclosure of any other particles meeting the definition of sLDL in the cited reference. As the reference does not disclose each and every limitation of the present claims, the reference does not anticipate the presently claimed methods of proliferating eukaryotic NSO cells.

In view of the above, Applicants respectfully request reconsideration and withdrawal of the rejection of claim 1 under 35 U.S.C. § 102(b).

The Rejection of the Claims as Being Obvious Should Be Withdrawn

Claim 13 was rejected under 35 U.S.C. § 103(a) for being unpatentable over Baillie *et al.* in view of Mainwaring and Wayte (U.S. Patent No. 7,258,998). Specifically, the Office Action concluded that it would have been obvious to one of ordinary skill in the art to use NSO cells as

disclosed in Mainwaring in place of the U937 cells described in Baillie *et al.* Claim 13 has been canceled as noted above. This rejection is respectfully traversed as it applies to amended claim 1.

Applicants respectfully note that a *prima facie* case of obviousness under 35 U.S.C. § 103(a) requires that a combination of references places the claimed subject matter in the public domain prior to Applicants' date of invention. See *In re Zenitz*, 333 F.2d 924, 142 USPQ 158 (C.C.P.A. 1964). Thus, establishing a *prima facie* case of obviousness requires that the cited references can be combined such that each and every element of the claimed invention is taught, explicitly or implicitly, by the references, and that a reasonable expectation of success exists in such a combination. As the Supreme Court has clarified, obviousness under § 103 still requires consideration of the factors set forth in *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966), including an analysis of the scope and content of the prior art and the difference between the claimed subject matter and the prior art. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 14 (2007).

As described above, Baillie *et al.* do not disclose methods of increasing proliferation of NSO cells as recited in the present claims. There is no teaching or suggestion in the reference that another cell type could be used according to the Baillie *et al.* methods. The results obtained, and the conclusions drawn, were specific to the U937 cell proliferation assay.

Applicants respectfully submit that disclosure of NSO cells in the Mainwaring *et al.* reference is insufficient to bridge the gap between Baillie *et al.* and the presently claimed invention. In particular, knowledge in the art that NSO cells are cholesterol dependent would have not provided one having ordinary skill in the art with a reasonable likelihood of success in developing methods of proliferating NSO cells comprising introducing sLDL particles to an NSO cell culture and allowing cells in the culture to proliferate in media lacking foetal calf serum. Mainwaring *et al.* do not teach or suggest supplementing serum-free media with cholesterol via sLDL particles. Furthermore, the cited reference even describes cholesterol-independent subspecies of NSO cell lines for which no cholesterol supplementation is required. See Mainwaring *et al.* at column 6, lines 56-60. Baillie *et al.* and Mainwaring *et al.*, either alone or in combination, fail to provide the skilled artisan with a reasonable likelihood of success in developing the presently claimed invention. As these cited references do not render obvious the

presently claimed methods, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a).

CONCLUSION

It is believed that the rejections of claims 1-3 and 11-12 have been overcome and the claims are in condition for allowance. Early notice to this effect is requested. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned agent.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 C.F.R. § 1.136(a), and any fee required therefore is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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